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ORIGINAL ARTICLE

# Downregulation of microRNA-217 and microRNA-646 acts as potential predictor biomarkers in progression, metastasis, and unfavorable prognosis of human osteosarcoma

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**Abstract** Despite the progress in therapeutic targets, it remains dissatisfactory for most osteosarcoma patients with metastasis or recurrence osteosarcoma. Therefore, it is required to determine the involved mechanisms of osteosarcoma. The aim of this study was to investigate the expression level of MiR-217 and miR-646 and also their association with clinicopathological features in patients with osteosarcoma. Total RNA was purified from patients with osteosarcoma and noncancerous bone tissues, and then quantitative real-time PCR was applied to evaluate the expression level of microRNAs. Our result suggested that miR-217 expression was remarkably decreased in osteosarcoma bone tissue when compared with noncancerous bone tissues (mean±SD 5.32±1.23, 2.[illegible]±0.78; $P$=0.024) and miR-646 expression decreased in osteosarcoma bone tissue in comparison with normal tissues (mean±SD 4.56±1.45, 1.76±1.24; $P$=0.041). Our findings indicated that decreased expression of MiR-217 and miR-646 was strongly correlated with high tumor, node, and metastasis (TNM) stage ($P$=0.015, $P$=0.002) and large cancer diameter ($P$=0.041, $P$=0.053). Kaplan-Meier survival and log-rank analysis indicated that shorter overall survival was strongly linked to decreased expression of miR-217 and miR-646 (log-rank test $P$=0.034, $P$=0.026). In terms of miR-217, multivariate Cox proportional hazards model analysis has showed that reduction of miR-217 expression ($P$=0.001), TNM stage ($P$=0.046), and lymph node metastasis ($P$=0.006) were independently linked to a short-time survival of patients. In terms of miR-646, low expression of miR-646 ($P$=0.021), TNM stage ($P$=0.052), and tumor size ($P$=0.043) were independently associated with poor survival of patients as prognostic factors. Our findings suggested that downregulation of MiR-217 and miR-646 was associated with progression of osteosarcoma. MiR-217 and miR-646 may play a key role in suppression of tumor in osteosarcoma and would be applied as a novel therapeutic agent.

**Keywords** Osteosarcoma · MiRNAs · Pathology · Metastasis · Patient

## Introduction

Osteosarcoma is known as the most common primary bone tumor in children and young adults [1, 2]. Despite the progress in therapeutic targets, it remains dissatisfactory for most osteosarcoma patients with metastasis or recurrence osteosarcoma. Furthermore, it is required to determine the involved mechanisms of osteosarcoma. Previous studies have indicated that microRNA expression may act as a significant marker for prognosis and detection of cancer. MicroRNAs are known as small noncoding RNAs [3–5] that act in many biological functions including cell fate specification, cellular proliferation, differentiation, and apoptosis through alteration of the targets expression by both downregulation and upregulation [3, 6, 7]. miRNAs are as either oncogenes or tumor suppressors in cancers of human [8].

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**Table 1** Correlation between microRNA expression and clinicopathological features of patients with osteosarcoma

| Characteristic | Number | miR-217 expression | | miR-646 expression | | *P* value (miR-217) | *P* value (miR-646) |
|---|---|---|---|---|---|---|---|
| | | Low | High | Low | High | | |
| Gender | | | | | | | |
| Male | 17 | 10 | 7 | 12 | 5 | 0.674 | 0.541 |
| Female | 24 | 17 | 12 | 15 | 9 | | |
| Age | | | | | | | |
| ≤60 | 13 | 8 | 5 | 7 | 4 | 0.563 | 0.512 |
| >60 | 28 | 11 | 17 | 13 | 15 | | |
| Tumor size (cm) | | | | | | | |
| ≤5 | 26 | 11 | 15 | 16 | 10 | 0.041 | 0.053 |
| >5 | 15 | 13 | 2 | 8 | 7 | | |
| Lymph node metastasis | | | | | | | |
| Negative | 34 | 16 | 19 | 14 | 20 | 0.126 | 0.102 |
| Positive | 7 | 4 | 3 | 5 | 2 | | |
| Histologic grade/differentiation | | | | | | | |
| Well and moderate | 19 | 11 | 8 | 13 | 6 | 0.243 | 0.214 |
| Poor | 22 | 13 | 9 | 12 | 10 | | |
| TNM stage | | | | | | | |
| I+II | 11 | 3 | 8 | 4 | 7 | 0.015 | 0.002 |
| III+IV | 30 | 24 | 6 | 22 | 8 | | |



It is worth noting that dysregulation of different microRNAs such as miR-34, miR-145, miR-206, miR-[illegible], miR-100, and miR-221 has recently been suggested in terms of osteosarcoma [7, 9–12]. These studies have reported that microRNAs can play their role as prognostic and diagnostic markers, as well as they indicated that microRNAs have potential therapeutic targets for osteosarcoma. Therefore, we evaluated the clinical significance of miR-217 and MiR-182 expression in human osteosarcoma and their association with clinicopathological features.

## Materials and methods

### Samples

A total of 41 samples were collected from patients with osteosarcoma and corresponding noncancerous bone tissue between September 2009 and April 2013 in different hospitals in Tehran, Iran. Patients underwent surgery without chemotherapy or radiotherapy. Before the surgery, informed consent was obtained from each patient. The specimens were stored at −80 °C until use. Moreover, the diagnosis and the histological grading were approved by pathologists. The clinicopathological features are classified in Table 1.

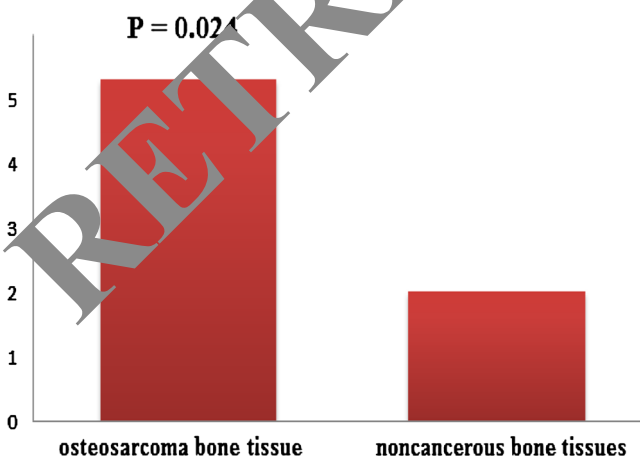


**Fig. 1** The relative expression level of MiR-217 expression between osteosarcoma bone tissue and noncancerous bone tissues

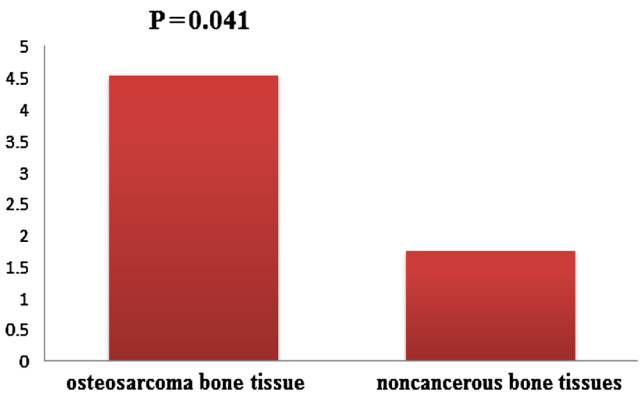


**Fig. 2** The relative expression level of MiR-646 expression between osteosarcoma bone tissue and noncancerous bone tissues

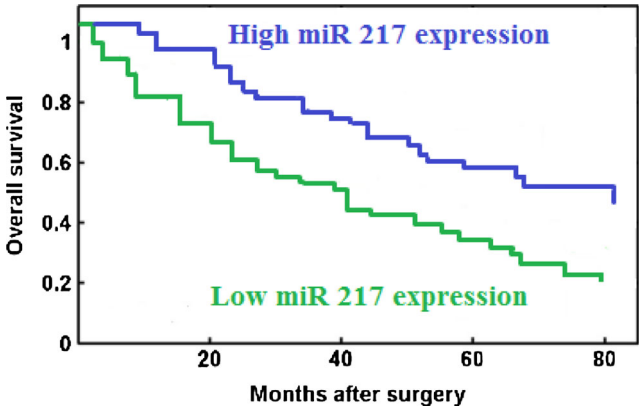


**Fig. 3** Correlation between miR-217 expression and survival time in patients with osteosarcoma

### Quantitative real-time PCR

In the present study, the total RNA was purified from samples of noncancerous bone tissue using TRIzol reagent based on the constructor's instructions for user. Gene-specific primers were used to synthesize cDNA from the TaqMan microRNA Assays and reagents from the TaqMan microRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Furthermore, real-time PCR was carried out using an Invitrogen kit by system of Rotor-gene 6000 (Qiagen). The primers were used from the TaqMan miRNA assays. Moreover, an internal standard control was applied (small nucleolar RNA U6). The ΔΔCt (ΔΔCt=$\Delta Ct_{\text{tumor samples}}-\Delta Ct_{\text{tu}}$ $_{\text{sample}}$) to qualify the expression rate of miR-217 a miR-1646.

### Statistical analysis

Obtained data were analyzed using SS 16.0 software (SPSS Inc., USA). Differences between all variables were evaluated using Student's *t* test or chi-square test. Moreover, survival

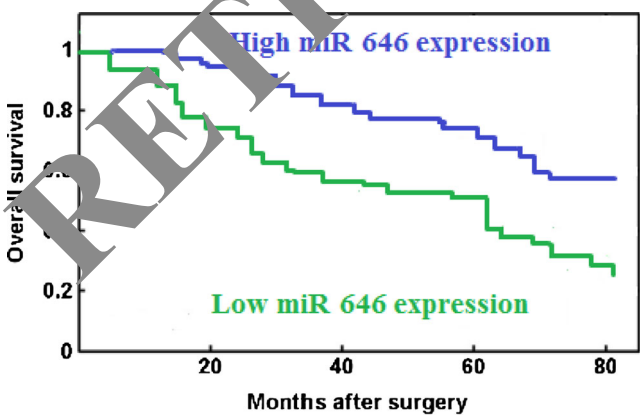


**Fig. 4** Correlation between miR-646 expression and survival time in patients with osteosarcoma

**Table 2** Multivariate analysis with a Cox proportional hazards model between miR-217 and clinicopathological factors

| Clinicopathological characteristics | HR | 95 % CI | *P* value |
|---|---|---|---|
| Gender | 0.742 | 0.672–2.341 | 0.623 |
| Age | 1.46 | 0.758–2.483 | 0.542 |
| TNM stage | 2.32 | 1.635–4.785 | 0.046 |
| Tumor size (cm) | 0.86 | 1.567–2.416 | 0.475 |
| Histological grading | 0.96 | 1.218–2.76 | 0.324 |
| Lymph node metastasis | 1.636 | 1.326–4.82 | 0.006 |
| miR-217 level | 2.724 | 1.619–5.427 | 001 |

evaluation was done by applying the log-rank test and Kaplan-Meier method. A Cox proportional hazards model was performed to assess multivariate analyses of prognostic values. Differences were statistically significant at $P<0.05$.

## Results

Our results suggested that miR-217 expression was remarkably deceased in osteosarcoma bone tissue when compared with noncancerous bone tissues (mean±SD 5.32±1.231, 2.01±0.78, $P=0.024$; Fig. 1), Furthermore, miR-646 expression was downregulated in osteosarcoma bone tissue in comparison with normal tissues (mean±SD 4.56±1.45, 1.76±1.24; $P=0.041$; Fig. 2). Osteosarcoma patients were categorized into two groups (low and high group) based on the median expression level of miR-217 and miR-646. The clinicopathological features of microRNAs in high and low expression groups were compared (Table 1).

The results of the current study indicated that decreased expression of MiR-217 and miR-646 was strongly correlated with high tumor, node, and metastasis (TNM) stage ($P=0.015$, $P=0.002$) and large cancer diameter ($P=0.041$, $P=0.053$). However, there were no significant relationships of MiR-217 and miR-646 expression with other factors including age ($P=0.563$, $P=0.512$), sex ($P=0.674$, $P=0.541$), anatomic location

**Table 3** Multivariate analysis with a Cox proportional hazards model between miR-646 and clinicopathological features

| Clinicopathological characteristics | HR | 95 % CI | *P* value |
|---|---|---|---|
| Gender | 0.638 | 0.364–2.721 | 0.562 |
| Age | 0.85 | 0.437–2.462 | 0.516 |
| TNM stage | 2.42 | 1.749–4.932 | 0.052 |
| Tumor size (cm) | 2.75 | 0.573–4.936 | 0.043 |
| Histological grading | 0.86 | 1.521–2.428 | 0.317 |
| Lymph node metastasis | 1.012 | 1.12–2.927 | 0.143 |
| miR-646 level | 2.823 | 1.624–5.424 | 0.021 |

(data not seen), ($P$=0.316, $P$=0.415), histologic grade/differentiation ($P$=0.243, $P$=0.214), and lymph node metastasis ($P$=0.126, $P$=0.102 (Table 1). Kaplan-Meier survival and log-rank analysis indicated that shorter overall survival was remarkably correlated with decreased expression of MiR-217 and miR-646 (log-rank test $P$=0.034, $P$=0.026; Figs. 3 and 4).

In terms of miR-217, multivariate Cox proportional hazards model analysis showed that low expression of miR-217 ($P$=0.001), TNM stage ($P$=0.046), and lymph node metastasis ($P$=0.006) was independently associated with poor survival of patients as prognostic factors (Table 2). In terms of miR-646, low expression of miR-646 ($P$=0.021), TNM stage ($P$=0.052), and tumor size ($P$=0.043) were independently associated with poor survival of patients as prognostic factors (Table 3).

## Discussion

MiRNAs are either oncogenes or tumor suppressors in human carcinogenesis [8]. Dysregulation of microRNAs has been previously reported in many kinds of tumor. Moreover, dysregulation of different miRNAs has been recently suggested in terms of osteosarcoma [7, 9–11]. It has been suggested that there is a correlation between miRNA expressions and tumor prognosis [13, 14]. Furthermore, determination of functional and clinical importance of a specific miRNA may provide effective management of the disease. In the current study, we evaluated the clinical significance of miR-217 and MiR-646 expression in human osteosarcoma.

Our result suggested that miR-217 expression was remarkably deceased in osteosarcoma bone tissue when compared with noncancerous bone tissues. This finding indicated that miR-217 can contribute to tumor occurrence and development. Dysregulation of miR-217 expression has been previously reported in much kind of human malignancies as a tumor suppressor. Zhao et al. indicated that miR-217 plays its role in the suppression of tumor by targeting the KRAS oncogene in pancreatic ductal adenocarcinoma [15]. On the other hand, upregulation of miR-217 was strongly reported in hepatocellular carcinoma (HCC) patients and also cell. Other study indicated that upregulation of the miR-217 could be linked to estrogen receptor status [16] and could have an important positive role in the progression of breast cancer. It can be interpreted that miR-217 may act as tissue specific. It has been suggested that miR-217 can be tumor specific and probably dependent on its targets in many kinds of cancer.

On the other hand, miR-646 expression was downregulated in osteosarcoma bone tissue in comparison with normal tissues in the current study. It has been reported that miR-646 downregulated in many cancer types [17], and increasing evidence suggests that it can play a key role as a tumor suppressor. Li et al suggested that miR-646 was decreased in renal cancer that was remarkably linked to metastasis of tumor via the MAPK pathway by targeting NOB1 [18]. Our result indicated that low expression of MiR-217 and miR-646 can be associated with tumor progression in osteosarcoma. Study results of Shen et al. demonstrate that miR-217 functions as a tumor-suppressive miRNA and inhibits the osteosarcoma tumorigenesis through targeting WASF3. In anoother study, Sun et al. demonstrated that miR-646 might be a tumor suppressor in osteosarcoma via the regulation of FGF2, which provided a potential prognostic biomarker and therapeutic target [19, 20].

The results of the current study indicated that decreased expression of MiR-217 and miR-646 was strongly correlated with high TNM stage and large tumor diameter that could be associated with tumor progression. Kaplan-Meier survival and log-rank analysis indicated that shorter overall survival was strongly linked to decreased expression of MiR-217 and miR-646, indicating that MiR-217 and miR-646 may be markers for prognosis in patients that suffered osteosarcoma.

In terms of miR-217, multivariate Cox proportional hazards model analysis showed that low expression of miR-217, TNM stage, and lymph node metastasis were independent prognostic factors for poor survival of patients. In terms of miR-646, low expression of miR-646, TNM stage, and tumor size were independently associated with poor survival of patients as prognostic factors.

In conclusion, our findings suggested that downregulation of miR-217 and miR-646 was associated with progression of osteosarcoma. MiR-217 and miR-646 may play a key role in suppression of tumor in osteosarcoma and likely would be applied as novel therapeutic agents.

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**Conflicts of interest** None

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